Genetics in Glaucoma

7

Mohana Sinnasamy, Murali Ariga, C. Praveena, and Sharmila Rajendrababu

7.1 Introduction

Glaucoma is a condition of progressive optic neuropathy with intraocular pressure (IOP) as a modifiable risk factor. As per the Global data for visual impairment (2002), Glaucoma was the second leading cause of blindness [1]. The knowledge of its pathogenesis can aid in the development of new therapeutic approaches and thereby reduction in blindness due to glaucoma.

The current management strategies are targeted towards reducing the secretion of aqueous, increasing the outflow facility or creating alternate drainage pathway. The evolving research on genetics of glaucoma and next generation sequencing is opening new insights into its pathogenesis and thereby new targets for the management.

7.2 Genes Involved in the Development of the Eye

The development of eye from the surface ectoderm, mesoderm and neural crest involves complex interactions of various growth factors with their receptors, signalling factors, transcription factors and the structural components that form the anatomy of the eye. The genes coding for these proteins can be broadly classified as:

M. Sinnasamy (✉) · C. Praveena
Swamy Eye Clinic, Chennai, Tamilnadu, India

M. Ariga
Department of Ophthalmology, Sundaram Medical Foundation, Chennai, Tamilnadu, India

S. Rajendrababu
Department of Glaucoma, Aravind Eye Hospital, Madurai, Tamilnadu, India
e-mail: shamsreddy@aravind.org

H. V. Nema, N. Nema (eds.), *Genetics of Ocular Diseases*,
https://doi.org/10.1007/978-981-16-4247-0_7

1. Structural genes form the cytoskeletal components and are responsible for the structural and biochemical functions
2. Regulatory genes regulate the expression of genes which code for proteins like transcription factors and signalling factors
3. Cell specific genes express cell specific proteins

In particular interest are the genes that contribute to the development of the anterior segment of the eye, i.e. the genes coding for the transcription factors like PITX2, PITX3, PAX6, FOXC1, FOXC2 and FOXC3 which have been frequently associated with anterior segment dysgenesis [2]. The abnormalities in the expression of these genes or abnormalities in the interaction between multiple gene products due to mutation can lead to structural and functional defects in the eye. For example, in transgenic mice, multiple factors like the cell signalling molecule, bone morphogenetic proteins and related signalling factors and their interaction have shown to be associated in the development of the anterior segment of the eye [3].

New molecules involved in the development of ocular structures are being identified. In a recent study, serine proteinase PRSS56 has been shown to play a role in the development and maintenance of ocular drainage tissues [4].

7.3 Discovering Candidate Genes for Glaucoma

It has been known that many types of glaucoma including primary open angle glaucoma, congenital glaucoma and developmental glaucoma run in families. Von Graefe identified multiple families with glaucoma occurring in many generations [5]. In addition, it has been observed that there is a higher concordance of glaucoma between mono zygotic than dizygotic twins. All these factors pointed to the fact that glaucoma is inherited at least in some proportion.

It is interesting to note that the elevation in IOP, reduced outflow facility and IOP rise in response to steroid administration have shown hereditary tendencies. Studies are being conducted in analysing a particular trait like IOP, CCT, etc. Such traits that influence the disease course and are known to have genetic component are called endophenotypes. The loci identified by genetic studies influencing a particular trait are called Quantitative trait loci (QTL).

Significant among such studies are the Beaver Dam study and Salisbury Eye Study which showed that elevated IOP is influenced by genes in seven loc on chromosomes 2, 5, 6, 7, 12, 15 and 19 and by environmental factors and that several optic disc parameters including vertical cup to disc ratio are heritable even more than the IOP [6, 7].

Another study, the Blue Mountain Eye Study showed that at least in 18% of patients with glaucoma, the IOP variance is genetically influenced [8]. A family study showed that chromosome 10q22 is associated with IOP in addition to a study of an affected sibling pair which showed that chromosome 5q22 and 14q22 also are associated with glaucoma [9, 10]. But the pattern of inheritance is elusive as they did not follow the laws of Mendelian Inheritance.

The genes involved in any disease can be identified and analysed by various methods like:

1. Linkage analysis: Analysing the pattern of inheritance of the identified gene in subsequent generations in families with the phenotype under study.
2. Association analysis: Analysing the contribution of a genetic variance or an environmental factor between case and control in a large cohort in causing the phenotype or a particular trait like IOP.
3. Genome Wide Association Studies (GWAS): A recently developed method which rapidly scans the genome of large study group for markers or variants of diseases, especially useful in analysing the diseases with low penetrance like POAG.

These approaches can also be used to study the pharmacogenetics (How an individual's body responds to a particular drug based on their genomic sequence?) and pharmacogenomics (How a drug responds to an individual based on his genomic sequence?) which will aid in individualised therapeutic approaches based on the genomic sequence of each individual in contrast to one treatment to all patients with same phenotype.

7.4 How Genes Cause a Disorder?

From the concept of single gene causing a rare disease, now we know that the genetic involvement is complex and multifactorial as follows:

1. Polygenic traits: Defects in multiple genes contribute to one disease. For example, Axenfeld–Rieger syndrome appears to be caused by at least three different genes located on chromosomes 4, 6 and 13 showing the genetic heterogeneity [11–13].
2. Single gene plus environmental factors: In exfoliative glaucoma which is associated with multiple polymorphisms of the LOXL1 gene, the genetic expression seems to be influenced by an environmental factor.
3. Single gene can be associated with multiple disorders, e.g. Rieger syndrome and iris hypoplasia can arise from mutations in the same gene on 4q25 (PITX2) and primary congenital glaucoma and iridogoniodysgenesis can be caused by mutations in the FKHL7 gene on 6p25 [14–16].
4. Incomplete penetrance: It is seen in cases where the gene is not expressed as phenotype in all individuals with the gene. For example, if an allele is present in 10 individuals and 7 express in their phenotype, the allele is said to have 70% penetrance as shown in Fig. 7.1.
5. Co dominant inheritance: Different genotypic combination of an allele causes a phenotype with characteristics of all the alleles in varying proportions and no allele is completely suppressed as shown in Fig. 7.2.

Fig. 7.1 Diagrammatic representation of incomplete penetrance—the allele is not translated to phenotype in all the individuals bearing the allele

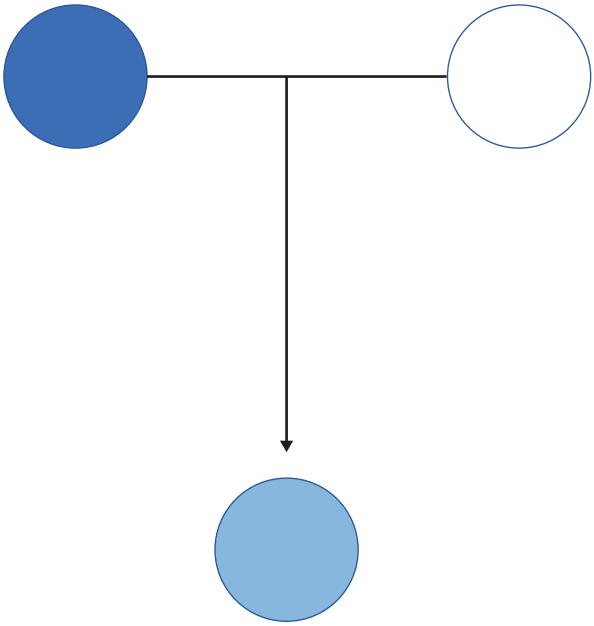

Fig. 7.2 Co-dominant inheritance—the offspring of two different alleles expresses a phenotype with characteristics of both the alleles

6. Imprinting effects: An epigenetic mechanism in which an allele of one parent is expressed and the other allele from other parent is imprinted possibly by post translational modifications of DNA like DNA methylation without altering the genetic sequence as shown in Fig. 7.3.
7. Mitochondrial inheritance

The pattern of inheritance seen in glaucoma varies with the type of glaucoma and is shown in Fig. 7.4.

7.5 Identification of Genes—Significance

The Human Genome Project has shown the possibility of decoding the entire sequence of the genome. A part of the Human Genome Project is the HapMap Project which catalogues the variances in the genome among individuals of diverse population. It has been identified that certain sequences of DNA variation are shared

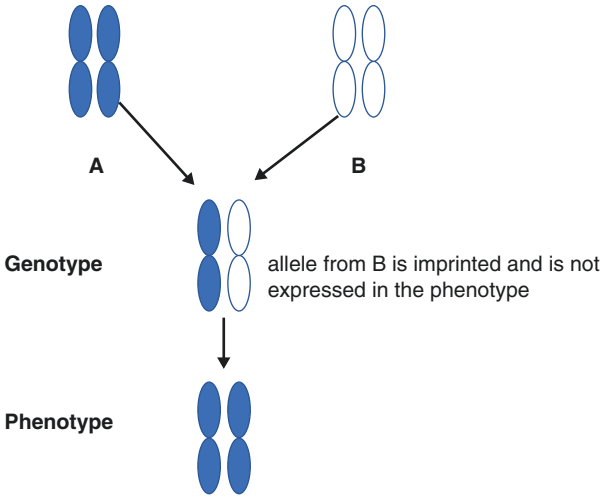

Fig. 7.3 Genomic Imprinting—one allele is imprinted by post translational modification in the offspring on a parent specific pattern

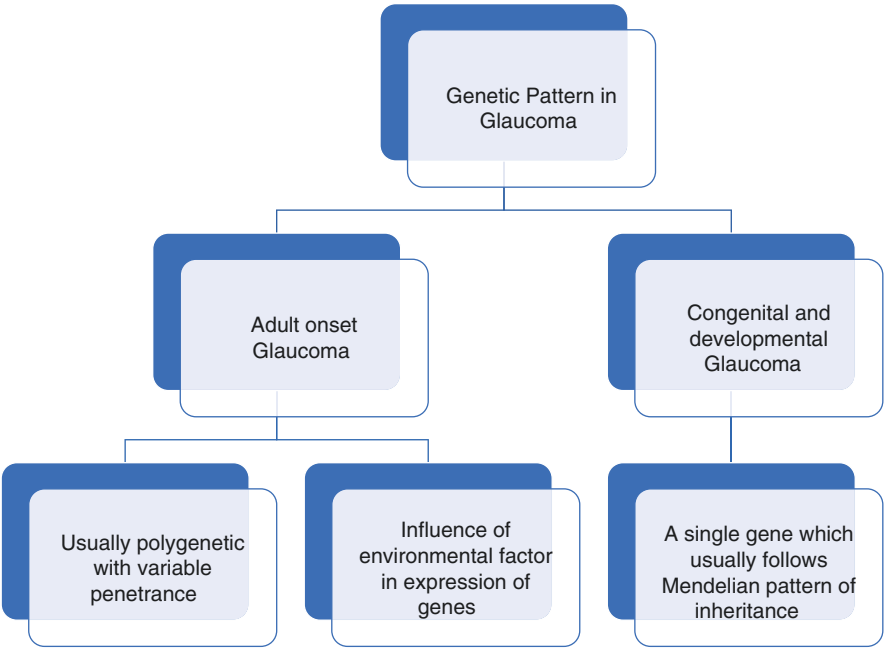

Fig. 7.4 The pattern of genetic inheritance in glaucoma follows the Mendelian pattern of inheritance in congenital and developmental glaucoma while the adult onset glaucoma exhibits genetic heterogeneity and environmental influence

among individuals of a population. They are called Single Nucleotide Polymorphisms (SNPs) and can be used as genetic markers. This can pave way to

1. Identify glaucoma even before its manifestation by the genetic analysis of the individuals with high risk.
2. To insert reparative genetic sequence through vectors like viruses in an individual with the faulty genes.
3. Selective embryo selection in couple with high risk genes thereby negating the possibility of inheriting the gene associated with glaucoma.

7.6 Genetic Nomenclature

The genes identified are given designation in a defined format by the HUGO (The Human Genome Organisation). It is usually in the following pattern: the first three letters denote the name of the disease—GLC indicates Glaucoma followed by a number 1,2 or 3 and an alphabet. (1—open angle, alphabet—identification of the gene.) For example: GLC1A, A refers to myocilin.

7.7 Primary Congenital Glaucoma (PCG)

It is a rare disease with the incidence of 1:10000 of which most of the cases are sporadic [17]. Around 10% cases are familial, and the inheritance is autosomal recessive. The gene frequently associated with congenital glaucoma is CYP1B1 and is related to the development of the eye. Although the exact mechanism is unknown, mice with mutant CYP1B1 gene had defective anterior chamber angle, increase in basal lamina and poorly developed Schlemm canal. This gene has been mapped in many linkage analyses of families with PCG. It encodes a protein of the cytochrome P450 family and is actively involved in the detoxification, xenobiotics, etc., which is highly expressed in foetal eyes. Its presence has been documented in corneal epithelium, keratocytes and iris stromal cells [18].

The identification of this gene led to search of factors that modify the expression of it. This search resulted in the identification of Tyrosinase gene which acts as a modifier gene, mutation of which lead to exacerbation of the defects in an individual with mutant CYP1b1. It was also interesting to note that administration of L-DOPA to these mutants circumvented the phenotypic defects showing that Tyrosinase pathway is involved in the development of anterior segment [19]. The locus for the gene CYP1B1 was mapped to the short arm of chromosome 2 in 2p21 [20]. In addition to this gene, further studies conducted on families with PCG have identified a second locus related to congenital glaucoma in the chromosome 1p36 [21]. The exact role of this locus remains to be analysed.

Extensive studies on CYP1B1 in Indian patients have shown various mutations—missense and termination mutations predominantly,although few deletions and frame shift mutations have also been observed [22]. The mutation that is observed consistently in an analysis of over 140 Indian families was Arg368His [23]

along with Pro129Leu, Glu229Lys, Arg390Cys, Gly61Glu and 367insA. Arg368His mutation is seen more frequently in South Indian population when compared to North Indian population, while the mutations Leu24Arg, Phe190Leu and Gly329Asp were observed in North Indian population [24].

LTBP2 (Latent Transforming Growth Factor Beta Binding Protein 2) Gene in chromosome 14q24 has been shown to be associated with PCG recently and is designated as GLC3C. This locus has been studied in Iranian population [25], Pakistani population and European gypsies [26]. It is intriguing to note that the specific mutation Arg299Stop in LTBP2 was found in both Pakistanis and European gypsies. It suggests that both these population have common ancestry as indicated by anthropological and genetic evidence.

7.8 Juvenile Onset Open Angle Glaucoma (JOAG)

It is characterised by early onset, high IOP and usually seen in myopes. The gene primarily associated with JOAG is TIGR (Trabecular Meshwork Induced Glucocorticoid Response gene),which is now referred as myocilin found in the chromosome 1q21-31. Around 10–20% of the JOAG patients have defective myocilin gene. At least five loci have been associated with JOAG including mutations like myocilin [27].

7.9 Primary Open Angle Glaucoma (POAG)

POAG is known to occur in more frequency with a positive family history and positive family history is an important risk factor for the development of primary open angle glaucoma as concluded by many studies including Barbados eye study and Baltimore eye studies. The screening of first-degree relatives of POAG in Indian population too has given similar results [28].

The genetic pattern of inheritance of this common type of glaucoma is complex and is associated with genetic heterogeneity. POAG exhibits variability in age of onset and is characterised by its low penetrance. The development of POAG is influenced by polygenic interactions and environmental factors and hence the inheritance pattern of POAG cannot be studied effectively by linkage analysis.

The gene that has been consistently shown to be involved in the pathogenesis of the POAG is MYOC gene identified in trabecular meshwork (earlier known as TIGR [29]). Mutations like Gly367Arg in MYOC gene cause reduced outflow through the trabecular meshwork (TM) due to aggregation of the mutant myocilin in the endoplasmic reticulum of meshwork cells [30, 31]. This causes defective secretion of the myocilin in the TM and thereby defective outflow through the TM. There have been various studies which have analysed the different mutations and polymorphisms in MYOC gene. The other mutations identified to be associated with POAG are OPTN gene coding for the protein Optineurin, WDR36,Neurotrophin 4 (NTF4),ankyrin repeat,TANK binding kinase 1(TBK1) and SOCS box-containing 10 [32].

In addition, genetic polymorphisms also play a role in POAG. Although they are not causative, the genetic polymorphism in ADRB1 and ADRB2 (the genes coding for β adrenergic receptors in trabecular meshwork and ciliary body) seems to play a role in the development of POAG and NTG in the Japanese population.

Given the heterogenous nature of genetic role in POAG, multiple genes have been identified by the Genome Wide Association studies(GWAS) which include Caveolin (CAV1/CAV2) [33], CDKN2B antisense RNA, TMCO1, ATOH 7, SIX1/SIX6, GAS7, chromosome 8q22, ABCA1, AFAP1, GMDS, PMM2, FNDC3B, TFGBR3, TXNRD2, ATXN2 and LRP12/ZFPM2 genes or actual loss of DNA(TBK1 and GALC). These are found in normal individuals but are found in high frequency in patients with POAG. Optineurin (GLC1E) gene in chromosome 10p15-14 plays a limited role specifically in familial and normal tension POAG. The genes associated with POAG are listed in Table 7.1. It is

Table 7.1 List of genes associated with POAG

Locus	Chromosome	Gene	Details of the study	Reference
GLC1A	1q31	MYOC	JOAG and adult onset POAG	Stone et al. [34]
GLC1B	2 cen-q13		Linkage analysis done in 6 families	Stoilova et al. [35]
GLC1C	3q21-24		A large family from North America	Wirtz et al. [36]
GLC1D	8q23		A family with POAG without mutations in 2 ce-q13 and 3q21-24	Trifan et al. [37]
GLC1E	10p15-14	OPTN	A British family with normal tension glaucoma	Sarfarazi et al. [38] and Rezaie et al. [39]
GLC1F	7q35-36	ASB10	A single family from Oregon, USA	Wirtz et al. [40] and Pasutto et al. [41]
GLC1G	5q22.1	WDR 36	Mapping of gene involved in T cell activation in seven families	Monemi et al. [42]
GLC1H	2p15-16	Yet to be identified	Seven families of POAG	Suriyapperuma et al. [43]
GLC1I	15q11-q13	Yet to be identified	Mapped in early adult onset POAG	Allingham et al. [44] and Wiggs et al. [45]
GLC1J	9q22	AD	In JOAG	Wiggs et al. [46]
GLC1K	20p12	AD	In JOAG	Wiggs et al. [46]
GLC1L	3p21-22	Yet to be identified	POAG in a Tasmanian family	Baird et al. [47]
GLC1M	5q22-31	Yet to be identified	JOAG in Filipino family studied in five generations	Pang et al. [48]
GLC1N	15q22-24	Yet to be identified	JOAG	Wang et al. [49]
GLC1O	19q13	NTF4	Normal tension and high tension POAG	Pasutto et al. [50]
GLC1P	12q14	AD	Normal tension glaucoma	Fingert et al. [51]

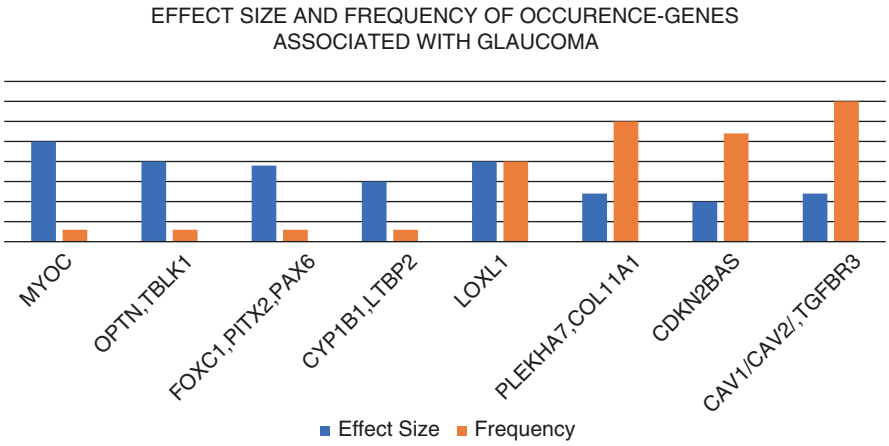

Fig. 7.5 Frequency and effect size of few genes associated with glaucoma. The genes with high effect size tend to occur rarely and the genes with low effect are seen more frequently

interesting to note that the genes with Mendelian form of inheritance (high effect) occur less frequently when compared to the genes with low effect size as shown in Fig. 7.5.

In South India, a study conducted to assess the MYOC mutations in Indian Population (107 subjects with POAG and 90 Normal subjects of a relatively unexplored ethnicity) found it in about 2% of the participants with POAG. Additionally, the study also analysed the type of mutations which showed Gly367 Arg and Thr 377Met was seen only in the POAG patients and not in normal controls. These mutations led to charged or bulky protein, defect in oligomer formation and poor secondary structure formation. Also, the non-sense mutation gln368stop frequently seen in Western Population was characteristically absent in Indian population [52].

Majority of the myocilin mutations are missense mutations (>80%) with few patients of frame shift and non-sense mutation (<5%) seen commonly in the sequence coding for olfactomedin like domain found in third exon of myocilin gene. These mutations have been documented in database (www.myocilin.com). The other common mutations seen in Indian populations are listed in Table 7.2.

Another possible mechanism for the aggregation of myocilin in primary open angle glaucoma could be due to variations in splicing of the protein structure. The mutations in the myocilin genomic region could result in synonymous codon changes or changes in the intron regions that may not change the amino acid sequence but may possibly cause variations in intron–exon splicing. The study done to analyse the possibility of polymorphism in the intronic region of the myocilin gene, showed that g.14072G>A polymorphism and g.1293C/T heterozygous polymorphism were present instead of the expected g.1293C/-polymorphism. Also,two new SNPs (g.1295G>T and g.1299T>G) and two previously reported SNPs (g. 1284G>T and g.1286G>T) were also identified [53].

Table 7.2 Common mutations in myocilin observed in Indian population

Reference	MYOC mutations identified	Method used
Mukhopadhyay et al. (2002)	Gln48His; Pro370Leu	Sequencing
Kanagavalli et al. (2003)	Gly367Arg, Thr377Met	Single Stranded Conformation Polymorphism—SSCP
SriPriya et al. (2004)	Gln48His	Sequencing
Chakrabarti et al. (2005)	Gln48His	PCR-RFLP and sequencing
Bhattacharjee et al. (2007)	Gln48His, Thr256Met, Thr353Ile, Pro370Leu, Gln368Stop, Gln399Asp, Ala427Thr	Sequencing
Kumar et al. (2007)	Gln48His	SSCP, PCR-RFLP and sequencing
Rose et al. (2007, 2011)	Ser331Thr, Pro370Leu, Gln48His, Thr353Ile/Asn480Lys	Sequencing and SSCP
Banerjee et al. (2012)	D273fsX344, Gln368Stop, Pro370Leu, Gly399Asp, Ala427Thr, Thr256Met, Ser331Leu	Sequencing

Table 7.3 Loci identified with the associated pathway in pathogenesis of POAG

Pathway	Loci associated
Cell division	CDKN2BAS, GAS7
Autophagy	CAV1,ABCA1GMDS,PMM2
Development	SIX6,FOXC1
Mitochondria	TXNRD2
TGF beta	CDKN2BAS, FNDC3B, TGFBR3
Lipids membranes	CAV1, ABCA1
Vascular tone	CAV1
Extracellular matrix	AFAP 1
CSF pressure	8q22

Another study on POAG was done for the clinical characterisation of a large POAG Pedigree (84 members of the identified family) along with genetic analysis of the participants for the genes myocilin, optineurin and TBK1. Interestingly, the participants did not harbour any of the three genes commonly associated with POAG [54]. This raises the need for further studies to identify responsible genes in different populations.

The genes identified in association with POAG contributing to various pathways of cell biology. Analysing their role in the pathways may shed more light on the pathogenesis of POAG. Some loci identified with the associated pathway in the pathogenesis of POAG are shown in Table 7.3.

Table 7.4 Identified Loci for physical traits of POAG

Factors	Qualitative trait loci
Intra ocular pressure	TMCO1,ABCA1
Central corneal thickness	Collagen Pathway
Optic disc size	ATOH7,CDC7
Vertical cup to disc ratio	CDKN2BAS, SIX1,SIX6

The International Glaucoma Genetic Consortium which strives to identify loci (QTL) related to individual physical trait and thereby the genes related to POAG through meta-analyses of many GWASes has identified qualitative trait loci of various features of POAG and are shown in Table 7.4.

7.10 Primary Angle Closure Glaucoma (PACG)

The incidence of primary angle closure glaucoma is high in certain regions of the world (East Asia) suggesting that PACG could be inherited at least in some proportion. It has been found that there is a sixfold higher chance of developing PACG in individuals with familial history. A study conducted with large number of subjects with family history of angle closure glaucoma or angle closure suspect to analyse the risk of developing angle closure glaucoma has shown that the risk is higher with history of PACG in the family when compared to an angle closure suspect [55]. This further emphasises the heritable nature of the traits. The physical traits like shallow anterior chamber trait was also observed to be running in families and is shown to be heritable in 70% of the individuals.

It has been known that CYP1B1 is associated with angle closure glaucoma in addition to primary open angle glaucoma and primary congenital glaucoma. A number of newer loci associated with PACG identified in GWAS conducted in 2012 and in 2016 with meta-analysis of GWAS are SNP-rs11024102 in PLEKHA7, rs1015213 in the intergenic sequence found between PCMTD1 and ST18 on chromosome 8q, rs3753841 in COL11A1, EPDR1, GLIS3, DPM2-FAM102, ChAT and FERMT2 [56, 57].

With multiple studies identifying an array of loci, the need for more studies to understand the role of these loci in the pathogenesis cannot be over emphasised. In a study from South India to detect three SNPs associated with PACG, only one was identified in the study population which highlights the need for studies with larger sample size to confirm the role of the other SNPs—PLEKHA7 and COL11A1 in the pathogenesis of PACG [58].

In case of nanophthalmos, wherein the affected individuals are susceptible to angle closure glaucoma, a locus NNO1 has been mapped to chromosome 11 in a family with high penetrance. The detailed analysis of this locus may shed further light in the pathogenesis of this condition.

Table 7.5 Pathways and the loci associated with PACG

Pathway	Loci associated
Collagen pathway	COL1A1
Cell adhesion	PLEKHA7,EPDR1, DPM2-FAM102A
Developmental gene—ACD	ABCC5
Cholinergic system	ChAT
Unknown function	ST18-PCMTD1

Like POAG, the loci identified with various pathways associated with PACG are shown in Table 7.5.

7.11 Developmental Glaucoma

Developmental glaucoma arise due to the defect in the morphogenesis of the anterior segment. The genes involved in the developmental glaucoma are those that are involved in the embryogenesis of the anterior segment of the eye as discussed earlier *vide supra* in the genes in development of the eye, i.e. the genes coding for transcription factors PITX2, PITX3 and FOXC1.

7.12 Pigmentary Glaucoma

Pigmentary glaucoma is known to follow autosomal dominant inheritance, commonly found in young myopic males. Like most types of glaucoma, this type also shows heterogenous genetic involvement. One locus has been identified in the chromosome 7q35-36. In mouse models, locus coding for the glycoprotein transmembrane protein Gpnmb has been associated with pigmentary dispersion. Further studies are needed to confirm the definite role of this locus.

7.13 Exfoliative Glaucoma

The exfoliative glaucoma involves deposition of microfibrillary material due to abnormal metabolism of extracellular material. The SNPs consistently associated with exfoliative glaucoma are found in the LOXL1 gene on the chromosome 15q24 coding for the Lysyl oxidase like 1 protein. This protein is involved in the formation of elastin. Multiple polymorphisms of this gene have been associated with exfoliative glaucoma like SNPs—rs2165241, rs1048661,rs3825942. All these polymorphisms have been detected in the first exon of LOXL1 gene.

It is interesting to note that population with the same genetic sequence of LOXL1 gene residing in different parts of the world have shown variations in their risk of developing exfoliative glaucoma. This shows that additional genetic or environmental factors may exist, which influence the expression of LOXL1 gene. Hence,

in-depth analysis of various factors can shed further light on the pathogenesis of exfoliative glaucoma.

Loci identified by the GWAS on exfoliative glaucoma different population, rs3825942 risk allele was found to be protective for South African population in contrast, the same allele was associated with risk of XFS in Caucasians. A rare allele p.Y407F of LOXL1 found in Japanese population has shown to be protective against XFS. This necessitates further analysis to identify how the individual susceptibility to the risk allele leads to the development of XFS.

7.14 Calcium Voltage-Gated Channel Subunit Alpha1 A (CACNA1A)

This locus identified in GWAS of XFS codes for the alpha 1A subunit of P/Q voltage dependent calcium channel and is seen to be distributed in different ocular tissues [59]. It is known that high calcium concentration is seen in the XFS fibrils and that calcium is needed for aggregation of fibrils. Whether altered calcium transport and its accumulation form the background for fibrillary deposition in XFS is to be studied. This raises the scope for newer therapy target for XFG. The various loci identified by the GWAS on exfoliative glaucoma are shown in Table 7.6.

The result of Genome Wide Association Study (GWAS) is based on the phenotype of the cases and controls participated in the study and the locus identified by a study involving larger sample size points to a greater association(relative risk attributable to the locus) with the disease or trait under study. If the participants under study exhibit a more defined phenotype, it aids in the deeper understanding of the function of the locus under study. For example, if we can study a large cohort of cases with exfoliative glaucoma associated with iris atrophy only or associated with lens subluxation only, the effects of that particular locus in the developing iris atrophy or lens subluxation can be studied in detail.

Table 7.6 Loci identified by the GWAS on exfoliative glaucoma [59]

Name of the locus	SNP/ Chromosome	Function
Proteasome maturation protein (Pomp)	rs7329408 on the Chr 13	Ubiquitin—proteasome synthesis
Transmembrane protein 136 (TMEM136)	rs11827818 on Chr 11	Unknown
1-acylglycerol-3-phosphate O-acyltransferase 1 I(AGPAT1)	rs3130283 on Chr 6	Located in MHC, it is involved in the synthesis of glycerolipids
RNA binding motif single stranded interacting protein 3 (RBMS3)	rs 12490863 on Chr 3	Shown to inhibit cell proliferation, angiogenesis and induce apoptosis and has tumour suppression properties
Semaphorin 6A	rs10072088 on Chr 5	Transmembrane protein

7.15 Limitations of GWAS

GWAS is a method of approach in analysing the genetic markers of many diseases in recent days. It is important that we realise it is not without limitations like:

1. It identifies only the signal, i.e. the region around the gene responsible and not the exact exon and hence can be taken as surrogate genetic marker only
2. Regulatory genetic sequences are often missed in the study.

Hence, the technology that can study the locus specifically can throw more light on the genetic pathways.

7.16 Genetics in Glaucoma—A Step in Future

7.16.1 Whole Exon/Genome Sequencing

The whole genome sequencing can bring out massive information on the functioning of each locus opening up a whole new therapeutic approach to our existing armamentarium. The high cost is the limiting factor at present but it may be more affordable in the future.

7.16.2 Comparative RNA Sequencing of Tissues

This technology analyses the RNA from the ocular tissues of the cases and control to identify the expression of the candidate gene or locus.

7.16.3 Gene Therapy

This therapeutic approach is based on altering the genetic sequence by replacing it or suppressing the candidate gene through the vectors like recombinant adeno-associated virus-rAAV, lentiviral vectors, etc.

The defective phenotype could also be modified by delivering the protein which is structurally and functionally designed to carry out the specific function. The ideal therapy would be cell specific without causing any toxicity or without eliciting unfavourable immune reaction and be able to address the symptoms in a single dose.

Eye being one of the accessible, immunologically unique and highly compartmentalised organ, it facilitates easy drug delivery and monitoring of the effects of the intervention. Also, the other eye can be ideal control for the intervention under study. Gene therapy has given promising results in retinal dystrophies like Leber's congenital amaurosis.

In glaucoma, retinal ganglion cells, trabecular meshwork and optic nerve head could be potential targets for the gene therapy. For example, BDNF (Brain Derived

Neurotrophic Factor) has neuroprotective properties and is being studied to reduce the retinal ganglion cell loss in glaucoma [60]. Also studies to increase the outflow in the trabecular meshwork through a specifically designed gene are being undertaken [61].

The knowledge of genetic role in glaucoma holds promises in greater understanding of the genesis of the disease, its molecular mechanisms and eventually leads to novel therapeutic approaches.

References

1. Resnikoff S, Pascolini D, Etyaolin D, et al. Global data on visual impairment in the year 2002. Bull World Health Organ. 2004;82:844–51.
2. Sowden JC. Molecular and developmental mechanisms of anterior segment dysgenesis. Eye (Lond). 2007;21(10):1310–8.
3. Wordinger RJ, Clark AF. Bone morphogenetic proteins and their receptors in the eye. Exp Biol Med (Maywood). 2007;232(8):979–92.
4. Labelle-Dumais C, Pyatla G, Paylakhi S, et al. Loss of PRSS56 function leads to ocular angle defects and increased susceptibility to high intraocular pressure. Dis Model Mech. 2020;13(5):dmm 042853. Published 2020 May 29.
5. von Graefe A. Pathology and treatment of glaucoma. Royal London Ophthalmic Hospital Rep J Ophthalmic Med Surg. 1871l Lon;7:108.
6. Duggal P, Klein AP, Lee KE, et al. A genetic contribution to intraocular pressure: the Beaver dam eye study. Investig Ophthalmol Vis Sci. 2005;46(2):555–60.
7. Klein BE, Klein R, Lee KE. Heritability of risk factors for primary open-angle glaucoma: the Beaver Dam Eye Study. Investig Ophthalmol Vis Sci. 2004;45:59.
8. Viswanathan AC, Hitchings RA, Indar A, et al. Commingling analysis of intraocular pressure and glaucoma in an older Australian population. Ann Hum Genet. 2004;68(Pt 5):489–97.
9. Charlesworth JC, Dyer TD, Stankovich JM, et al. Linkage to 10q22 for maximum intraocular pressure and 1p32 for maximum cup-to-disc ratio in an extended primary open-angle glaucoma pedigree. Investig Ophthalmol Vis Sci. 2005;46(10):3723–9.
10. Rotimi CN, Chen G, Adeyemo AA, et al. Genome wide scan and fine mapping of quantitative trait loci for intraocular pressure on 5q and 14q in West Africans. Investig Ophthalmol Vis Sci. 2006;47(8):3262–7.
11. Semina EV, Reiter R, Leysens NJ, et al. Cloning and characterization of a novel bicoid-related homeobox transcription factor gene, RIEG, involved in Rieger syndrome. Nat Genet. 1996;14:392–9.
12. Mirzayans F, Gould DB, Heon E, et al. Axenfeld-Rieger syndrome resulting from mutation of the FKHL7 gene on chromosome 6p25. Eur J Hum Genet. 2000;8(1):71–4.
13. Phillips JC, del Bono EA, Haines JL, et al. A second locus for Rieger syndrome maps to chromosome 13q14. Am J Hum Genet. 1996;59(3):613–9.
14. Héon E, Sheth BP, Kalenak JW, et al. Linkage of autosomal dominant iris hypoplasia to the region of the Rieger syndrome locus. Hum Mol Gene. 1995;4:1435–9.
15. Nishimura D, Swiderski R, Alward W, et al. The fork-head transcription factor gene FKHL7 is responsible for glaucoma phenotypes which map to 6p25. Nat Genet. 1998;19:140–7.
16. Mears AJ, Jordan T, Mirzayans F, et al. Mutations of the fork-head/winged-helix gene, FKHL7, in patients with Axenfeld-Rieger anomaly. Am J Hum Genet. 1998;63:1316–28.
17. Ho CL, Walton DS. Primary congenital glaucoma: 2004 update. J Pediatr Ophthalmol Strabismus. 2004;41(5):271–88.
18. Zhao Y, Sorenson CM, Sheibani N. Cytochrome P4501B1 and primary congenital glaucoma. J Ophthalmic Vis Res. 2015;10:60–7.
19. Libby RT, Smith RS, Savinova OV, et al. Modification of ocular defects in mouse developmental glaucoma models by tyrosinase. Science. 2003;299:1578–81.

20. Sarfarazi M, Akarsu AN, Hossain A, Turacli ME, Aktan SG, Barsoum-Homsy M, et al. Assignment of a locus (GLC3A) for primary congenital glaucoma (Buphthalmos) to 2p21 and evidence for genetic heterogeneity. Genomics. 1995;30(2):171–7.
21. Akarsu AN, Turacli ME, Aktan SG, et al. A second locus (GLC3B) for primary congenital glaucoma (Buphthalmos) maps to the 1p36 region. Hum Mol Genet. 1996;5(8):1199–203.
22. Panicker SG, Reddy ABM, Mandal AK, Ahmed N, Nagarajaram HA, Hasnain SE, Balasubramanian D. Identification of novel mutations causing familial primary congenital glaucoma in Indian pedigrees. Investig Ophthalmol Vis Sci. 2002;43(5):1358–66.
23. Reddy ABM, Panicker SG, Mandal AK, Hasnain SE, Balasubramanian D. Identification of R368H as a predominant CYP1B1 allele causing primary congenital glaucoma in Indian patients. Investig Ophthalmol Vis Sci. 2003;44:4200–3.
24. Reddy ABM, Kaur K, Mandal AK, Panicker SG, Thomas R, Hasnain SE, et al. Mutation spectrum of the CYP1B1 gene in Indian primary congenital glaucoma patients. Mol Vis. 2004;10:696–702.
25. Narooie-Nejad M, Paylakhi SH, Shojaee S, Fazlali Z, Rezaei Kanavi M, Nilforushan N, et al. Loss of function mutations in the gene encoding latent transforming growth factor beta binding protein 2, LTBP2, cause primary congenital glaucoma. Hum Mol Genet. 2009;18(20):3969–77.
26. Ali M, McKibbin M, Booth A, Parry DA, Jain P, Riazuddin SA, et al. Null mutations in LTBP2 cause primary congenital glaucoma. Am J Hum Genet. 2009;84(5):664–71.
27. Sud A, Del Bono EA, Haines JL, Wiggs JL. Fine mapping of the GLC1K juvenile primary open-angle glaucoma locus and exclusion of candidate genes. Mol Vis. 2008;14:1319–26.
28. Rajendrababu S, Gupta N, Vijayakumar B, Kumaragurupari R, Krishnadas SR. Screening first degree relatives of persons with primary open angle glaucoma in India. J Curr Glaucoma Pract. 2014;8(3):107–12.
29. Polansky JR, Fauss DJ, Chen P, et al. Cellular pharmacology and molecular biology of the trabecular meshwork inducible glucocorticoid response gene product. Ophthalmologica. 1997;211(3):126–39.
30. Kanagavalli J, Pandaranayaka E, Krishnadas SR, et al. In vitro and in vivo study on the secretion of the Gly367Arg mutant myocilin protein. Mol Vision. 2007;13:1161–8.
31. Kanagavalli J, Pandaranayaka E, Krishnadas SR, Krishnaswamy S, Sundaresan P. A review of genetic and structural understanding of the role of myocilin in primary open angle glaucoma. Indian J Ophthalmol. 2004;52(4):271–80.
32. Abu-Amero K, Kondkar AA, Chalam KV. An updated review on the genetics of primary open angle glaucoma. Int J Mol Sci. 2015;16(12):28886–911.
33. Wiggs JL, Kang JH, Yaspan BL, et al. Common variants near CAV1 and CAV2 are associated with primary open- angle glaucoma in Caucasians from USA. Hum Mol Genet. 2011;20:4707–13.
34. Stone EM, Fingert JH, Alward WL, Nguyen TD, Polansky JR, Sunden SL, et al. Identification of a gene that causes primary open angle glaucoma. Science. 1997;275:668–70.
35. Stoilova D, Child A, Trifan OC, Crick RP, Coakes RL, Sarfarazi M. Localization of a locus (GLC1B) for adult-onset primary open angle glaucoma to the 2cen-q13 region. Genomics. 1996;36(1):142–50.
36. Wirtz MK, Samples JR, Kramer PL, Rust K, Topinka JR, Yount J, et al. Mapping a gene for adult-tonset primary open-angle glaucoma to chromosome 3q. Am J Hum Genet. 1997;60:296–304.
37. Trifan OC, Traboulsi EI, Stoilova D, Alozie I, Nguyen R, Raja S, et al. A third locus (GLC1D) for adult-onset primary open-angle glaucoma maps to the 8q23 region. Am J Ophthalmol. 1998;126(1):17–28.
38. Sarfarazi M, Child A, Stoilova D, Brice G, Desai T, Trifan OC, et al. Localization of the fourth locus (GLC1E) for adult-onset primary open-angle glaucoma to the 10p15-p14 region. Am J Hum Genet. 1998;62(3):641–52.
39. Rezaie T, Child A, Hitchings R, Brice G, Miller L, Coca-Prados M, et al. Adult-onset primary open-angle glaucoma caused by mutations in optineurin. Science. 2002;295:1077–9.
40. Wirtz MK, Samples JR, Rust K, Lie J, Nordling L, Schilling K, et al. GLC1F, a new primary openangle glaucoma locus, maps to 7q35-q36. Arch Ophthalmol. 1999;117:237–41.

41. Pasutto F, Keller KE, Weisschuh N, Sticht H, Samples JR, Yang YF, et al. Variants in ASB10 are associated with open-angle glaucoma. Hum Mol Genet. 2012;21(6):1336–49.
42. Monemi S, Spaeth G, DaSilva A, Popinchalk S, Ilitchev E, Liebmann J, et al. Identification of a novel adult-onset primary open-angle glaucoma (POAG) gene on 5q22.1. Hum Mol Genet. 2005;14(6):725–33.
43. Suriyapperuma SP, Child A, Desai T, Brice G, Kerr A, Crick RP, et al. A new locus (GLC1H) for adult-onset primary open-angle glaucoma maps to the 2p15-p16 region. Arch Ophthalmol. 2007;125:86–92.
44. Allingham RR, Wiggs JL, Hauser ER, Larocque-Abramson KR, Santiago-Turla C, Broomer B, et al. Early adult-onset POAG linked to 15q11-13 using ordered subset analysis. Investig Ophthalmol Vis Sci. 2005;46(6):2002–5.
45. Wiggs JL, Allingham RR, Hossain A, Kern J, Auguste J, DelBono EA, et al. Genome-wide scan for adult onset primary open angle glaucoma. Hum Mol Genet. 2000;9(7):1109–17.
46. Wiggs JL, Lynch S, Ynagi G, Maselli M, Auguste J, Del Bono EA, et al. A genome-wide scan identifies novel early-onset primary open-angle glaucoma loci on 9q22 and 20p12. Am J Hum Genet. 2004;74(6):1314–20.
47. Baird PN, Foote SJ, Mackey DA, Craig J, Speed TP, Bureau A. Evidence for a novel glaucoma locus at chromosome 3p21-22. Hum Genet. 2005;117(217):249–57.
48. Pang CP, Fan BJ, Canlas O, Wang DY, Dubois S, Tam PO, et al. A genome-wide scan maps a novel juvenile-onset primary open angle glaucoma locus to chromosome 5q. Mol Vis. 2006;12:85–92.
49. Wang DY, Fan BJ, Chua JK, Tam PO, Leung CK, Lam DS, et al. A genome-wide scan maps a novel juvenile-onset primary open-angle glaucoma locus to 15q. Investig Ophthalmol Vis Sci. 2006;47(12):5315–21.
50. Pasutto F, Matsumoto T, Mardin CY, Sticht H, Brandsttool JH, Michels-Rautenstrauss K, et al. Heterozygous NTF4 mutations impairing neurotrophin-4 signalling in patients with primary open-angle glaucoma. Am J Hum Genet. 2009;85(4):447–56.
51. Fingert JH, Robin AL, Stone JL, Roos BR, Davis LK, Scheetz TE, et al. Copy number variations on chromosome 12q14 in patients with normal tension glaucoma. Hum Mol Genet. 2011;20(12):2482–9.
52. Kanagavalli J, Pandaranayaka E, Krishnadas SR, et al. Evaluation and understanding of myocilin mutations in Indian primary open angle glaucoma patients. Mol Vision. 2003;9:606–14.
53. Pandaranayaka PJ, Prasanthi N, Kannabiran N, et al. Polymorphisms in an intronic region of the myocilin gene associated with primary open-angle glaucoma--a possible role for alternate splicing. Mol Vis. 2010;16:2891–902. Published 2010 Dec 29.
54. Kader MA, Namburi P, Ramugade S, et al. Clinical and genetic characterization of a large primary open angle glaucoma pedigree. Ophthalmic Genet. 2017;38(3):222–5.
55. Kavitha S, Zebardast N, Palaniswamy K, et al. Family history is a strong risk factor for prevalent angle closure in a South Indian population. Ophthalmology. 2014;121(11):2091–7.
56. Vithana EN, Khor CC, Qiao C, et al. Genome-wide association analyses identify three new susceptibility loci for primary angle closure glaucoma. Nat Genet. 2012;44(10):1142–6.
57. Khor CC, Do T, Jia H, et al. Genome-wide association study identifies five new susceptibility loci for primary angle closure glaucoma. Nat Genet. 2016;48(5):556–62.
58. Duvesh R, Verma A, Venkatesh R, et al. Association study in a South Indian population supports rs1015213 as a risk factor for primary angle closure. Investig Ophthalmol Vis Sci. 2013;54:5624–8.
59. Prof. Tin Aung, Singapore National Eye Centre, National University of Singapore- Proceedings of All India Ophthalmology Conference 2020.
60. Osborne A, Khatib TZ, Songra L, et al. Neuroprotection of retinal ganglion cells by a novel gene therapy construct that achieves sustained enhancement of brain-derived neurotrophic factor/tropomyosin-related kinase receptor-B signalling. Cell Death Dis. 2018;9(10):1007.
61. Konda SM, Kiland JA, Mohr M, et al. Schlemmath Dis. of retinal ganglion asurement, catheterization and substance delivery in live monkeys. Investig Ophthalmol Vis Sci July. 2019;60:1788.